

In the Specification

Please substitute the following paragraphs:

[0074] 1 µg of RNA was incubated in a final reaction medium of 10 µl in the presence of 5 U of T₄ phage RNA ligase in the buffer provided by the manufacturer (Gibco – BRL), 40 U of the RNase inhibitor ~~RNasin~~ RNASIN (Promega) and, 2 µl of ³²pCp (Amersham #PB 10208).

[0077] 0.1 OD unit of either a capped oligoribonucleotide of 47 nucleotides (including the cap) or an uncapped oligoribonucleotide of 46 nucleotides were treated as follows. The oligoribonucleotides were produced by in vitro transcription using the transcription kit ~~“AmpliScribe T7”~~ AMPLISCRIBE T7 (Epicentre Technologies). As indicated below, the DNA template for the RNA transcript contained a single cytosine. To synthesize the uncapped RNA, all four NTPs were included in the in vitro transcription reaction. To obtain the capped RNA, GTP was replaced by an analogue of the cap, m⁷G(5')ppp(5')G. This compound, recognized by polymerase, was incorporated into the 5' end of the nascent transcript during the step of initiation of the transcription but was not capable of incorporation during the extension step. Consequently, the resulting RNA contained a cap of its 5' end. The sequences of the oligoribonucleotides produced by the in vitro transcription reaction were:

+Cap:

5'm⁷GpppGCAUCCUACUCCCAUCCAAUUCCACCCUAACUCCUCC
CAUCUCCAC-3' (SEQ ID NO:1)

-Cap:

5'-

pppGCAUCCUACUCCCAUCCAAUUCCACCCUAACUCCUCCCAUC
UCCAC-3' (SEQ ID NO:2)

[0110] 10 ml of ACA34 GEL ~~AcA34~~ (BioSeptra#230151) gel were equilibrated in 50 ml of a solution of 10 mM Tris pH 8.0, 300 mM NaCl, 1 mM EDTA, and 0.05% SDS. The mixture was allowed to sediment. The supernatant was eliminated and the gel was resuspended in 50 ml of buffer. This procedure was repeated 2 or 3 times.

[0146] Following second strand synthesis, the ends of the cDNA were blunted with T4 DNA polymerase (Biolabs) and the cDNA was digested with EcoRI. Since methylated dCTP was used during cDNA synthesis, the EcoRI site present in the tag was the only site which was hemi-methylated. Consequently, only the EcoRI site in the oligonucleotide tag was susceptible to EcoRI digestion. The cDNA was then size fractionated using exclusion chromatography (AcA, Biosepra). Fractions corresponding to cDNAs of more than 150 bp were pooled and ethanol precipitated. The cDNA was directionally cloned into the SmaI and EcoRI ends of the phagemid ~~pBlueScript~~ PBLUESCRIPT vector (Stratagene). The ligation mixture was electroporated into bacteria and propagated under appropriate antibiotic selection.

[0150] Plasmid inserts were first amplified by PCR on PE 9600 thermocyclers (Perkin-Elmer), using standard SETA-A and SETA-B primers (Genset SA), ~~AmpliTaqGold~~ AMPLITAQGOLD (Perkin-Elmer), dNTPs (Boehringer), buffer and cycling conditions as recommended by the Perkin-Elmer Corporation.

[0151] PCR products were then sequenced using automatic ABI Prism 377 sequencers (Perkin Elmer, Applied Biosystems Division, Foster City, CA). Sequencing reactions were performed using PE 9600 thermocyclers (Perkin Elmer) with standard dye-primer chemistry and ~~ThermoSequenase~~ THERMOSEQUENASE (Amersham Life Science). The primers used were either T7 or 21M13 (available from Genset SA) as appropriate. The primers were labeled with JOE, FAM, ROX, and TAMRA dyes. The dNTPs and ddNTPs used in the sequencing reactions were purchased from Boehringer. Sequencing buffer, reagent concentrations and cycling conditions were as recommended by Amersham.

[0158] Programs which may be used to search or compare the stored sequences include the ~~MacPattern~~ MACPATTERN (EMBL), BLAST, and BLAST2 program series (NCBI), basic local alignment search tool programs for nucleotide (BLASTN) and peptide (BLASTX) comparisons (Altschul et al, **J. Mol. Biol.** **215**: 403 (1990)) and FASTA (Pearson and Lipman, **Proc. Natl. Acad. Sci. USA**, **85**: 2444 (1988)). The BLAST programs then extend the alignments on the basis of defined match and mismatch criteria.

[0173] To extend the analysis of the reliability of the procedures for isolating 5' ESTs from ESTs in the NETGENE™ database, a similar analysis was conducted using a database composed of human mRNA sequences extracted from ~~GenBank~~ GENBANK database release 97 for comparison. For those 5' ESTs derived from mRNAs included in the ~~GeneBank~~ GENBANK database, more than 85% had their 5' ends close to the 5' ends of the known sequence. As some of the mRNA sequences available in the ~~Genbank~~ GENBANK database are deduced from genomic sequences, a 5' end matching with these sequences will be counted as an internal match. Thus, the method used here underestimates the yield of ESTs including the authentic 5' ends of their corresponding mRNAs.

[0209] The method takes advantage of the known 5' sequence of the mRNA. A reverse transcription reaction is conducted on purified mRNA with a poly 14dT primer containing a 49 nucleotide sequence at its 5' end allowing the addition of a known sequence at the end of the cDNA which corresponds to the 3' end of the mRNA. For example, the primer may have the following sequence: 5'-ATC GTT GAG ACT CGT ACC AGC AGA GTC ACG AGA GAG ACT ACA CGG TAC TGG TTT TTT TTT TTT TTVN -3' (SEQ ID NO: 14). Those skilled in the art will appreciate that other sequences may also be added to the poly dT sequence and used to prime the first strand synthesis. Using the primer and a reverse transcriptase such as the ~~Superscript II~~ SUPERSCRIPT II (Gibco BRL) or ~~Rnase H Minus M-MLV~~ RNASE H MINUS M-MLV (Promega) enzyme, a reverse transcript anchored at the 3' polyA site of the RNAs is generated.

[0210] After removal of the mRNA hybridized to the first cDNA strand by alkaline hydrolysis, the products of the alkaline hydrolysis and the residual poly dT primer are eliminated with an exclusion column such as an ~~AeA34~~ ACA34 (Biosepra) matrix as explained in Example 11.

[0214] The first PCR run of 25 cycles is performed using the ~~Advantage Tth Polymerase Mix~~ ADVANTAGE TTH POLYMERASE MIX (Clontech) and the outer primer from each of the nested pairs. A second 20 cycle PCR using the same enzyme and the inner primer from each of the nested pairs is then performed on 1/2500 of the first PCR product. Thereafter, the primers and nucleotides are removed.

[0219] Sequencing of extended cDNAs can be performed using a Die Terminator approach with the ~~AmpliTaq~~ AMPLITAQ DNA polymerase FS kit available from Perkin Elmer.

[0265] The cDNA codes of SEQ ID NOS. 40-84 and 130-154 or the polypeptide codes of SEQ ID NOS. 85-129 and 155-179 may be stored and manipulated in a variety of data processor programs in a variety of formats. For example, the cDNA codes of SEQ ID NOS. 40-84 and 130-154 or the polypeptide codes of SEQ ID NOS. 85-129 and 155-179 may be stored as text in a word processing file, such as MicrosoftWORD or WORDPERFECT or as an ASCII file in a variety of database programs familiar to those of skill in the art, such as DB2, SYBASE, or ORACLE. In addition, many computer programs and databases may be used as sequence comparers, identifiers, or sources of reference nucleotide or polypeptide sequences to be compared to the cDNA codes of SEQ ID NOS. 40-84, and 130-154 or the polypeptide codes of SEQ ID NOS. 85-129 and 155-179. The following list is intended not to limit the invention but to provide guidance to programs and databases which are useful with the cDNA codes of SEQ ID NOS. 40-84 and 130-154 or the polypeptide codes of SEQ ID NOS. 85-129 and 155-179. The programs and databases which may be used include, but are not limited to: ~~MaePattern~~ MACPATTERN (EMBL), ~~DiscoveryBase~~ DISCOVERYBASE (Molecular Applications Group), ~~GeneMine~~ GENEMINE (Molecular Applications Group), ~~Look~~ LOOK (Molecular Applications Group), ~~MaeLook~~ MACLOOK (Molecular Applications Group), BLAST and BLAST2 (NCBI), BLASTN and BLASTX (Altschul et

al, *J. Mol. Biol.* 215: 403 (1990)), FASTA (Pearson and Lipman, *Proc. Natl. Acad. Sci. USA*, 85: 2444 (1988)), FASTDB (Brutlag et al. *Comp. App. Biosci.* 6:237-245, 1990), ~~Catalyst~~ CATALYST (Molecular Simulations Inc.), ~~Catalyst~~ CATALYST/SHAPE (Molecular Simulations Inc.), ~~Cerius².DBAccess~~ CERIUS².DBACCESS (Molecular Simulations Inc.), ~~HypoGen~~ HYPOGEN (Molecular Simulations Inc.), ~~Insight II~~ INSIGHT II, (Molecular Simulations Inc.), ~~Discover~~ DISCOVER (Molecular Simulations Inc.), ~~CHARMm~~ CHARMM (Molecular Simulations Inc.), ~~Felix~~ FELIX (Molecular Simulations Inc.), ~~DelPhi~~ DELPHI, (Molecular Simulations Inc.), ~~QuanteMM~~ QUANTEM, (Molecular Simulations Inc.), ~~Homology~~ HOMOLOGY (Molecular Simulations Inc.), ~~Modeler~~ MODELER (Molecular Simulations Inc.), ISIS (Molecular Simulations Inc.), ~~Quanta/Protein Design~~ QUANTA/PROTEIN DESIGN (Molecular Simulations Inc.), ~~WebLab~~ WEBLAB (Molecular Simulations Inc.), ~~WebLab Diversity Explorer~~ WEBLAB DIVERSITY EXPLORER (Molecular Simulations Inc.), ~~Gene Explorer~~ GENE EXPLORER (Molecular Simulations Inc.), ~~SeqFold~~ SEQFOLD (Molecular Simulations Inc.), the EMBL/Swissprotein database, the MDL Available Chemicals Directory database, the MDL Drug Data Report data base, the Comprehensive Medicinal Chemistry database, ~~Derwent's~~ DERWENT'S World Drug Index database, the ~~BioByteMasterFile~~ BIOBYTEMASTERFILE database, the ~~Genbank~~ GENBANK database, and the ~~Genseq~~ GENSEQ database. Many other programs and data bases would be apparent to one of skill in the art given the present disclosure.

[0353] One useful expression vector for generating β -globin chimerics is pSG5 (Stratagene), which encodes rabbit β -globin. Intron II of the rabbit β -globin gene facilitates splicing of the expressed transcript, and the polyadenylation signal incorporated into the construct increases the level of expression. These techniques as described are well known to those skilled in the art of molecular biology. Standard methods are published in methods texts such as Davis et al., (**Basic Methods in Molecular Biology**, L.G. Davis, M.D. Dibner, and J.F. Battey, ed., Elsevier Press, NY, 1986) and many of the methods are available from Stratagene, Life Technologies, Inc., or Promega. Polypeptide may additionally be produced from the construct using in vitro translation systems such as the ~~In-vitro ExpressTM~~ IN VITRO EXPRESSTM Translation Kit (Stratagene).

[0408] Proteins which interact with the polypeptides encoded by extended cDNAs or portions thereof, such as receptor proteins, may be identified using two hybrid systems such as the ~~Matchmaker Two Hybrid System 2~~ MATCHMAKER TWO HYBRID SYSTEM 2 (Catalog No. K1604-1, Clontech). As described in the manual accompanying the ~~Matchmaker Two Hybrid System 2~~ MATCHMAKER TWO HYBRID SYSTEM 2 (Catalog No. K1604-1, Clontech), which is incorporated herein by reference, the extended cDNAs or portions thereof, are inserted into an expression vector such that they are in frame with DNA encoding the DNA binding domain of the yeast transcriptional activator GAL4. cDNAs in a cDNA library which encode proteins which might interact with the polypeptides encoded by the extended cDNAs or portions thereof are inserted into a second expression vector such that they are in frame with DNA encoding the activation domain of GAL4. The two expression plasmids are transformed into yeast and the yeast are plated on selection medium which selects for expression of selectable markers on each of the expression vectors as well as GAL4 dependent expression of the HIS3 gene. Transformants capable of growing on medium lacking histidine are screened for GAL4 dependent lacZ expression. Those cells which are positive in both the histidine selection and the lacZ assay contain plasmids encoding proteins which interact with the polypeptide encoded by the extended cDNAs or portions thereof.

[0463] In a preferred embodiment, chromosomal localization of an extended cDNA (or genomic DNA obtainable therefrom) is obtained by FISH as described by Cherif et al. (*Proc. Natl. Acad. Sci. U.S.A.*, **87**:6639-6643, 1990). Metaphase chromosomes are prepared from phytohemagglutinin (PHA)-stimulated blood cell donors. PHA-stimulated lymphocytes from healthy males are cultured for 72 h in RPMI-1640 medium. For synchronization, methotrexate (10 μ M) is added for 17 h, followed by addition of 5-bromodeoxyuridine (5-BudR, 0.1 mM) for 6 h. Colcemid (1 μ g/ml) is added for the last 15 min before harvesting the cells. Cells are collected, washed in RPMI, incubated with a hypotonic solution of KCl (75 mM) at 37°C for 15 min and fixed in three changes of methanol:acetic acid (3:1). The cell suspension is dropped onto a glass slide and air dried. The extended cDNA (or genomic DNA obtainable therefrom) is labeled with biotin-16 dUTP by nick translation according to the manufacturer's instructions (Bethesda Research Laboratories, Bethesda, MD), purified using a ~~Sephadex~~ SEPHADEX G-50 column (Pharmacia, Upssala, Sweden)

and precipitated. Just prior to hybridization, the DNA pellet is dissolved in hybridization buffer (50% formamide, 2 X SSC, 10% dextran sulfate, 1 mg/ml sonicated salmon sperm DNA, pH 7) and the probe is denatured at 70°C for 5-10 min.

[0541] As shown by the alignment in Figure 10 between the protein of SEQ ID NO:120 and PLM, the amino acid residues are identical except for positions 3 and 5 in the 92 amino acid long matched protein. The substitution of a proline residue at position 3 par another neutral residue, serine, is conservative. In addition, the protein of the invention also exhibits the typical ATP1G /PLM/MAT8 PROSITE signature (position 27 to 40 in bold in Figure 10) for a family containing mostly proteins known to be either chloride channels or chloride channel regulators. In addition, the protein of invention contains 2 short transmembrane segments from positions 1 to 21 and from 37 to 57 as predicted by the software ~~TopPred II~~ TOPPRED II (Claros and von Heijne, *CABIOS applic. Notes*, **10** :685-686 (1994)). The first segment (in italic) corresponds to the signal peptide of PLM and the second transmembrane domains (underlined) matches the transmembrane region (double-underlined) shown to be the chloride channel itself (Chen *et al.*, *Circ. Res.*, **82**:367-374 (1998)).